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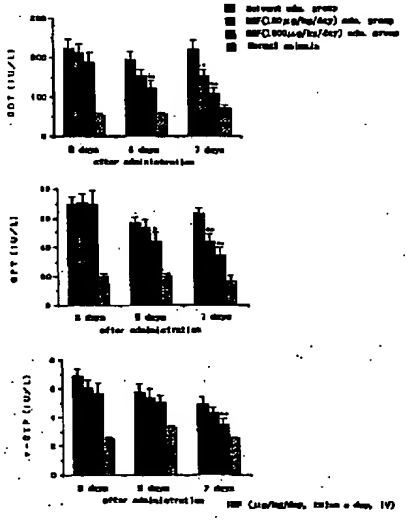
(54) COLLAGEN HYDROLYSIS ACCELERATOR

(57) A collagen hydrolysis accelerator and a fibrosis remedy each containing hepatocyte growth factors (HGF) as the active ingredient. As the HGF can accelerate collagen hydrolysis (collagenase activity increase) to thereby effectively cure fibrosis, it is possible to prevent or treat diseases caused by a lowered collagenase activity and the fibrosis characterized by the excessive fibroblast production of connective tissue matrix.

Fig. 4



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Description

Technical Field

The present invention relates to an agent accelerating collagen decomposition and a therapeutic agent for fibrosis disorder. More particularly, the present invention relates to an agent accelerating collagen decomposition and a therapeutic agent for fibrosis disorder comprising HGFs (Hepatocyte Growth Factors) as an active ingredient.

Background Art

Fibrosis is a disease characterized by the excessive accumulation of a connective tissue component, and one which is a noticeable component in fibrosis is collagen. Accumulation of collagen occurs in a variety of viscera, for example, brings about pulmonary fibrosis in lung and liver fibrosis in liver. Also in skin, for example, the accumulation of collagen brings about disorders such as cutis keloid formation.

In many cases, the net accumulation of collagen in fibrosis is the result of disproportion between factors which bring about decomposition and production of collagen.

Though various medications have been conducted to treat the disorder and sickness of fibrosis, they were mainly for symptomatic therapy for the disorder in general, and were not those which aim at dissolving pathogenesis, namely the disproportion between metabolism factors which regulate decomposition and production of collagen and the other connective tissue component. Therefore, in these therapies, there was no especially effective method in point of tissue repair. For example, though local corticosteroid was used to treat the initial inflammation stage of cut is keloid formation and success was made to a certain extent, such steroid treatment has little or no effect on the latter fibrosis stage such as in case of actual formation of keloid as a result of excessive production of collagen.

As described above, in the prior art, there could not have been discovered a safe and effective method which treats fibrosis disorder of human, inhibits more formation of fibrous tissue and reduces or completely removes the focus of fibrosis previously formed.

The object to be solved by the present invention is to provide an agent accelerating collagen decomposition which can induce the decomposition of a collagen matrix of excessively accumulated connective tissue in tissue and a therapeutic agent useful for treatment of fibrosis disorder.

Disclosure of the Invention

The present inventors have intensively investigated to solve the object described above. As a result, the inventors found that HGFs have an action to promote the decomposition of collagen and are effective for the treatment of fibrosis disorder based on their action. And thereby the present invention has been completed.

Namely, the present invention is an agent accelerating collagen decomposition containing at least one of HGFs as an active ingredient.

Another invention is a therapeutic agent for fibrosis disorder containing at least one of HGFs as an active ingredient.

Still other inventions are a method for accelerating collagen decomposition comprising administering at least one of HGFs in an effective amount; a method for treating fibrosis disorder comprising administering at least one of HGFs in an effective amount; use of at least one of HGFs for producing an agent accelerating collagen decomposition; and use of at least one of HGFs for producing a therapeutic agent for fibrosis disorder.

Brief Description of the Drawings

Fig. 1 is a chart which shows medicine-administration schedule in Example 1.

Fig. 2 is a chart which shows medicine-administration schedule in Test A of Example 2.

Fig. 3 is a chart which shows medicine-administration schedule in Test B of Example 2.

Fig. 4 is a graph which shows the results of GOT, GPT and γ-GTP measurements in Test A of Example 2.

Fig. 5 is a graph which shows the results of GOT, GPT and hepaplastein tests and the measurement of liver Hyp (hydroxyproline) content in Test B of Example 2.

Fig. 6 is a graph which shows the survival effect of HGF on a liver fibrosis of rats in Test A of Example 3. In Fig. 6, the dotted line indicates a group (control group, n=10) receiving physiological saline, the broken line indicates a group (n=5) receiving HGF at a dose of 50μg/kg body weight, and the solid line indicates a group (n=5) receiving HGF at a dose of 200 μg/kg body weight.

Fig. 7 is a photograph which shows the liver-fibrosis relieving effect of HGF on rats with liver fibrosis in Test B of Example 3.

Fig. 8 is a graph which shows the proportion (%) of fibrous tissue of an each individual liver in Example 4.

Best Modes of Carrying Out th Invention

In the present invention, the term HGFs indicate proteins which exhibit hepatocyte growth activity, and HGF (Hepatocyte Growth Factor) and the like are exemplified.

As the HGFs used in the present invention, compounds prepared by various methods can be used if they are purified to an extent that they may be used as a medicine.

Many methods are known to prepare HGFs, and, for example, HGF can be obtained by extraction and purification from organs such as liver, spleen, lung, bone marrow, brain, kidney, placenta and the like, blood cells such as platelets, leukocytes and the like, plasma and serum of mammals such as rat, cow, horse, sheep and the like (FEBS Letters, 224, 312, 1987; Proc. Natl. Acad. Scl. USA. 86, 5844, 1989).

Also, it is possible to obtain HGF by cultivation of primary culture cells or cell lines producing HGF, followed by separation and purification from the culture product (e.g. culture supernatant, cultured cell, etc.). Further, HGF can be obtained by gene engineering method which comprises cloning the gene encoding HGF, inserting it into a suitable vector, transfecting the vector to obtain a transformant, and isolating the aimed recombinant HGF from the culture supernatant of the transformant (e.g. Nature, 342, 440, 1989; Biochem. Biophys. Res. Commun., 163, 967, 1989). The host cell is not specifically limited, and various host cells conventionally used in gene engineering methods can be used, which are, for example, Escherichia coli, Bacillus subtilis, yeast, filamentous fungi, and plant or animal cells.

More concretely, the method of extracting and purifying HGF from fresh tissues is, for example, to administer carbon tetrachloride to a rat intraperitoneally, remove a liver from the rat with hepatitis, homogenize it, and purify by the ordinary protein purification techniques such as gel column chromatography using S-Sepharose and heparin Sepharose, HPLC and the like.

Further, using the gene engineering method, the gene encoding the amino acid sequence of human HGF is inserted into a vector such as bovine papilloma virus DNA and the like to obtain an expression vector, by using this expression vector, animals cells such as Chinese hamster ovary (CHO) cells, mouse C127 cells, monkey COS cells and the like are transformed, and HGF can be obtained from the culture supernatant of the transformants.

As to HGFs thus obtained, there are possibilities that a part of the amino acid sequence of HGFs will be deleted or substituted with other amino acid(s), that another amino acid sequence is partially inserted, that one, two or more amino acids are attached to the C and/or N terminals, or that sugars are similarly deleted or substituted.

The agent accelerating collagen decomposition of the present invention comprises the above-described HGFs as an active ingredient, and the HGFs have accelerating action for collagen decomposition (increase of collagenase activity) as shown in the test examples mentioned later. Therefore, the agent accelerating collagen decomposition of the present invention is effective for the treatment of the following fibrosis disorder as well as the prevention thereof, and useful for the treatment and prevention of a disease due to reduced collagenase activity, for example, osteopetrosis or the like.

Further, the therapeutic agent for fibrosis disorder of the present invention likewise comprises the above-described HGFs as an active ingredient, and is useful for the treatment of the fibrosis disorders characterized by excessive production of fibroblast-derived connective tissue matrix containing collagen, fibronectin and glycosaminoglycan (GAG). They include the following disorders.

Arterial sclerosis, chronic glomerulonephritis, cutis keloid formation, progressive systemic sclerosis (PSS), liver fibrosis, pulmonary fibrosis, cystic fibrosis, chronic graft versus host disease, scleroderma (local and systemic), Peyronie's disease, penis fibrosis, urethrostenosis after the test using a cystoscope, inner accretion after surgery, myelofibrosis, idiopathic retroperitoneal fibrosis.

The agent accelerating collagen decomposition and the therapeutic agent for fibrosis disorder of the present invention are applicable to the treatment of fibrosis disorder and the acceleration of collagen decomposition in mammals (for example, cow, horse, pig, sheep, dog, cat and the like) in addition to human.

The agent accelerating collagen decomposition and therapeutic agent for fibrosis disorder of the invention may be prepared in various preparation forms (for example, liquid, tablet, capsule), and generally it is prepared in the form of injection, inhalation, suppository or oral preparation containing HGFs as the active ingredient alone or together with conventionally used carrier. The injection may be prepared by the conventional method, and for example, HGF is dissolved in a proper solvent (for example, sterilized water, buffer solution, physiological saline), filtered and sterilized, and put in a container aseptically. The content of HGF in the injection may be usually 0.0002 to 0.2 w/v%, preferably 0.001 to 0.1 w/v%. As the oral preparation, it is manufactured in various preparation forms, including tablet, granule, fine granule, powder, soft or hard capsule, liquid, emulsion, suspension or syrup, and these preparations may be manufactured by the conventional method. As to the suppository, it can be prepared by the conventional pharmaceutical method using a suppository base (for example, cacao oil, laurin oil, glycero-gelatin, macrogol, witepsol, etc.). Further, as to the inhalation, it also can be prepared by the conventional pharmaceutical method. The HGFs content in the preparation may be properly adjusted depending on the preparation form and the disease to be treated.

In production of the preparation, it is preferable to add a stabilizer, and examples of the stabilizer include albumin, globulin, gelatin, glycine, mannitol, glucose, dextran, sorbitol, ethylene glycol and the like. Moreover, the preparation of

the invention may contain other additives necessary for pharmaceutical preparation, such as an excipient, a dissolving aid, an antioxidant, a pain-alleviating agent, an agent for isotonicity and th like. In liquid preparation, it is preferabl to store it under frozen conditions or after the removal of water by a process such as lyophilization. The lyophilized preparation is used by dissolving again in distilled water for injection and the like before use.

The agent accelerating collagen decomposition and therapeutic agent for fibrosis disorder of the invention are administered through various routes depending on the preparation form. For example, the injection is administered by intravenous, intraarterial, subcutaneous, intramuscular and the like. The dose is adjusted properly depending on symptoms, age and body weight of patient, and generally 0.05 mg to 500 mg, preferably 1 mg to 100 mg of HGFs is administered once or several times per day.

Industrial Applicability

HGFs, the active ingredients of the present invention, accelerate the decomposition of collagen (increase of collagenase activity), and can effectively treat fibrosis disorder. Therefore, according to the present invention, the prevention and treatment of a disease due to reduced collagenase activity (for example, osteopetrosis or the like) and the above-described fibrosis disorders characterized by excessive production of fibroblast-derived connective tissue matrix are possible.

Examples

The present invention is illustrated in detail with reference to the following Preparation Examples and Examples, which should not be construed as limiting the scope the present invention. Preparation Example 1

Production example of HGF formulation

(1)

HGF	20μg
Human serum albumin	100mg

The above-described substances were dissolved in 0.01 M of PBS (pH 7.0), and the resulted solution was filled up to 20 ml with the same solvent. The resulting mixture was sterilized, then 2 ml aliquots of the mixture were poured into vials separately and they were lyophilized and sealed.

(2)

HGF	40μg
Tween 80	1mg
Human serum albumin	100mg

The above-described substances were dissolved in a physiological saline for injection to prepare a mixture having a total volume of 20 ml. The resulting mixture was sterilized, then 2 ml aliquots of the mixture were poured into vials separately and they were lyophilized and sealed.

Example 1

Preventing and improving effects of HGF on liver fibrosis of rats induced with dimethylnitrosamine

1. Test method

(1) Animals used: Wistar male rat, 5 weeks old

(2) Test schedule

Dimethylnitrosamine (DMN) was administered intraperitoneally to rats at a dose of 10μl/kg on every Tuesday, Wednesday, Thursday over 4 weeks. HGF was administered intravenously to the rats at a dose of 500μg/kg twice a day (1000μg/kg/day) for 28 days from the first DMN administration. The administration schedule is shown in Fig. 1. On the 29th day, the test rats were subjected to the following measurement.

(3) Measurement

The rats were dissected to measure the weight of the liver. Further, the hydroxyproline content (Hyp; an index for fibrosis) and collagenase (collagen decomposition enzyme) activity in liver tissue were measured respectively by the method of Kivirikko et al. (Anal. Biochem, 19, 249, 1967) and the method of Murawaki et al. (J. Biochem, 108 , 241, 1990). Further, DNA and protein contents in liver tissue were measured respectively by the Dishe method modified by Burton (Biochem, J, 62, 315, 1956) and Protein assay kit (manufactured by Bio-Rad Ltd.). The results are shown in Table 1.

Also, blood was collected from postcava at the same time, and the clinical biochemical test of the collected serum was analyzed by a Hitachi 7150 type automatic analysis apparatus. Then, using the EDTA-added blood collected from postcava, platelet number, leucocyte number, erythrocyte number, hematocrit value, hemoglobin concentration were measured by a multi item automatic blood cell counting apparatus (E-4000, manufactured by Sysmex). Also, using the plasma obtained by mixing 3.8 % aqueous sodium citrate solution and the blood collected from posteava in a ratio of 1:9, the plasma coagulation ability (prothrombin time, fibrinogen content, coagulation time by the hepaplastein test and the thrombo test) was measured by an automatic coagulation ability measuring apparatus (KC-40). The result is shown in Table 2.

Table 1

	Solvent administered group	HGF administered group	normal animals
Liver weight (g)	9.33 ± 0.72	13.02 ± 0.53**	13.59 ± 0.51**
Total DNA content (mg/liver)	33.6 ± 2.5	39.3 ± 1.8*	44.8 ± 1.7**
Total protein content (g/liver)	1.36 ± 0.12	1.84 ± 0.09**	2.33 ± 0.09**
Collagenase activity (μg/min/g-liver)	0.22 ± 0.01	0.36 ± 0.07**	0.27 ± 0.02
Hydroxyproline content (μg/g-liver)	423.1 ± 35.9	300.1 ± 18.0**	129.3 ± 6.4**
Average ± standard error (n = 10)			

* : $P < 0.05$,
** : $P < 0.01$ vs. solvent administered group

Table 2

Serum biochemistry test valu	Solvent administered group	HGF administered group	normal animals
GOT (IU/l)	136 ± 9	78 ± 4**	64 ± 3**
GPT (IU/l)	50 ± 4	28 ± 1**	15 ± 1**
γ-GTP (U/l)	4.8 ± 0.4	3.4 ± 0.2**	1.9 ± 0.1**
Total bilirubin (mg/dl)	0.45 ± 0.08	0.25 ± 0.01**	0.19 ± 0.01**
Direct bilirubin (mg/dl)	0.20 ± 0.02	0.18 ± 0.00	0.13 ± 0.01**
Total protein (g/dl)	4.9 ± 0.1	6.4 ± 0.1**	5.7 ± 0.0**
Albumin (g/dl)	2.4 ± 0.1	3.1 ± 0.1**	2.6 ± 0.0**
Blood sugar (mg/dl)	131 ± 4	152 ± 6**	180 ± 8**
Total cholesterol (mg/dl)	53 ± 2	98 ± 5**	72 ± 3**
HDL-cholesterol (mg/dl)	28.0 ± 1.9	61.6 ± 3.9**	41.3 ± 1.7**
Triglyceride (mg/dl)	70 ± 9	152 ± 16**	157 ± 16**
Phospholipid (mg/dl)	116 ± 4	208 ± 8**	160 ± 5**
β-lipoprotein (mg/dl)	107 ± 11	222 ± 20**	202 ± 18**
Blood, coagulation test value	Solvent administered group	HGF administered group	normal animals
Platelet number (10^4/μl)	31 ± 5	78 ± 4**	105 ± 5**
Leukocyte number (10^2/μl)	144 ± 6	101 ± 8**	87 ± 8**
Erythrocyte number (10^4/μl)	667 ± 16	702 ± 9**	755 ± 6**
Hematocrit value (%)	39.9 ± 0.9	41.6 ± 0.3*	44.3 ± 0.3**
Hemoglobin concentration (g/l)	12.7 ± 0.3	13.6 ± 0.1**	14.6 ± 0.1**
Prothrombin time (sec)	15.6 ± 0.5	13.8 ± 0.2*	13.8 ± 0.2*
Fibrinogen (g/dl)	1.45 ± 0.10	2.05 ± 0.11**	2.31 ± 0.05**
Hepaplastein time (sec)	37.3 ± 2.7	28.6 ± 0.6**	28.5 ± 0.5**
Thrombo test time (sec)	30.0 ± 1.9	22.5 ± 0.3**	22.8 ± 0.3**
Average ± standard error (n = 10)			

* : $P < 0.05$,
** : $P < 0.01$ vs. solvent administered group

2. Result

As shown in Table 1 and Table 2, owing to repeated administration of DMN, remarkable progression of the liver fibrosis and shrink of the liver were observed in the solvent administered group (control), and clear depression in the liver function was confirmed by the clinical biochemistry, blood and coagulation test values. On the other hand, the liver function test values of the HGF administered group manifested values near normal animals with significant difference as compared with those values of the solvent administered group, and revealed clear improving effect. Further, because of HGF administration, collagenase activity, protein and DNA content in the liver tissue significantly increased, the hydroxyproline content which is the index of fibrosis significantly decreased, and the liver weight was recovered to approximately normal level.

Example 2

Action of HGF on liver fibrosis of rats induced with carbon tetrachloride

Carbon tetrachloride was administered orally to Wister male rats (6 weeks old) at a dose of 0.7 ml/kg on every Monday and Thursday for 12 weeks to prepare liver fibrosis models. These rats with liver fibrosis induced with carbon tetrachloride were subjected to the following two tests.

(1) Test A

Repeated administration test

HGF was administered intravenously to the above-described rats with liver fibrosis induced with carbon tetrachloride (one group includes 13-14 individuals) at doses of 50 and 500μg/kg twice a day (100 and 1000μg/kg/day) for 7 days. The administration schedule is shown in Fig. 2. The changes of GOT, GPT and γ-GTP values in the serum on day 3, 5 and 7 after the initiation of HGF administration were compared with those in the serum of solvent administered group (control). The results are shown in Fig. 4.

As shown in Fig.4, accelerating effect of HGF on recovery was observed on day 5 at a dose of 1000μg/kg/day and on day 7 at a dose of 100μg/kg/day.

(2) Test B

Drip injection test

HGF was infused into the above-described rats with liver fibrosis induced with carbon tetrachloride (n=12-13 per group) at doses of 100 and 1000μg/kg/day through a catheter indwelt in cervical vein, and they were dissected 72 hours after the initiation of HGF infusion. The HGF infusion schedule is shown in Fig. 3.

GOT, GPT, serum total protein, albumin in the serum were measured by a Hitachi 7150 type automatic analysis apparatus, and with respect to the coagulation ability of the plasma obtained by mixing 3.8 % aqueous sodium citrate solution and the blood collected from postcava in a ratio of 1:9, the coagulation time in the hepaplastein test was measured by an automatic coagulation ability measuring apparatus (KC-40). Further, the hydroxyproline content in liver tissue (Hyp) which is the index of fibrosis was measured by the above-described method of Kivirikko et al. The results are shown in Table 3 and Fig. 5.

As shown in Table 3 and Fig. 5, when HGF was administered at a dose more than 100μg/kg/day, there were observed the improvement of the liver function test value, the recovery in hypoproteinemia and reduction of the hydroxyproline content in liver tissue which indicates improvement of fibrosis, each depending on the doses.

Table 3

	Total protein conc. (g/dl)	Albumin conc. (g/dl)
Solvent administered group	5.0 ± 0.1	1.8 ± 0.1
HGF administered group (100 μg/kg/day)	5.5 ± 0.2*	2.1 ± 0.1*
HGF administered group (1000 μg/kg/day)	5.7 ± 0.2**	2.1 ± 0.1*
Healthy and normal animals	6.0 ± 0.1**	2.4 ± 0.03**
Average ± standard error (n = 12 to 13)		

*: $P < 0.05$,

**: $P < 0.01$ vs. solvent administered group

Exampl 3

Effect of HGF on liver fibrosis of rats induced with DMN and on accompanied liver function insufficiency

(1) Test A

The HGF-administered group and HGF-unadministered group of the rats with liver fibrosis induced with DMN were tested for their survival ratios.

The administration schedule of medicines is shown in upper portion of Fig. 6. DMN dissolved in a physiological saline in a concentration of 1 % was administered intraperitoneally to SD male rats three times a week for 6 weeks as indicated by arrows at a dose of 10μl of DMN per 1kg body weight. The human HGF or the physiological saline was administered intravenously to rats every day for the period shown by a hatched band from the 21st day after the initiation of DMN administration, to examine the time course of survival rates of tested rats. The results are shown in Fig. 6. In the Fig. 6, the dotted line indicates the physiological saline administered group (control group, n=10), the broken line indicates a HGF 50μg/kg body weight administered group (n=5), and the solid line indicates a HGF 200μg/kg body weight administered group (n=5).

As shown in Fig. 6, by the administration of HGF, the survival rates were improved, and especially, no death was observed in the HGF 200 μg/kg body weight administered group.

(2) Test B

The type I collagen deposition (histological observation) of the liver of the DMN administered rat was examined under administration or non-administration of HGF.

Namely, for detection of collagen fibers, the rats were sacrificed and the livers were taken out on the 42nd day in the tests shown in Fig. 6. For the immunofluorescent stain of collagen, the liver was quickly frozen in OTC compound, and the prepared slices were reacted with a rabbit anti rat collagen type I antibody (obtained from LSL Ltd.) and a fluorescent-labeled goat anti rabbit IgG antibody, then the specimens were photographed. The results are shown in Fig. 7.

As shown in Fig. 7, there was a significant difference between administration and non-administration of HGF in depositions of type I collagen in the liver. In control rat group administrated the physiological saline, the deposition of type I collagen in the liver was evident, thick or thin bundles of a type I collagen fiber were deposited in the wide range around blood vessels and hepatocytes.

These events disappeared by the administration of HGF in a dose-dependent manner, the improvement of lobulus structure was remarkable in the HGF administered group. In this way, the preventing effect of HGF on liver fibrosis was demonstrated by the histological observation of the liver section.

(3) Test C

The changes in prothrombin time, hepatocyte-leaked enzyme and hepatic hydroxyproline contents by HGF administration were examined.

Namely, rats were treated according to the experiment scheme shown in Fig. 6. Prothrombin time (PT), values in serum (plasma) of albumin (Alb), glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT) and alkaline phosphatase (ALP), and liver hydroxyproline content (HYP) were measured respectively in a normal rat group (n=5. indicated as normal rat in Table 4), a group in which DMN treatment had been conducted for 5 weeks and only physiological saline had been administered (n=5, indicated as 5W in Table 4), a group in which DMN treatment had been conducted for 6 weeks and only physiological saline had been administered (n=5, indicated as 6W in Table 4) or groups in which HGF was administered at doses of 50μg/kg body weight (n=3, indicated as 50 in Table 4) and 200μg/kg body weight (n=5, indicated as 200 in Table 4) instead of the physiological saline. The results are shown in Table 4. Here, the values indicated as 5W were obtained by sacrificing rats on the 35th day, and the other values were obtained by sacrificing rats on the 42nd day after the initiation of the DMN treatment.

As shown in Table 4, owing to HGF administration, it was noticed that the liver fibrosis and liver function insufficiency were improved.

Table 4

			PT (sec.)	Alb (mg/dl)	GOT (IU/L)	GPT (IU/L)	ALP (IU/L)	HYPRO (μg/g Liver)
Normal rat		(n=5)	14.2 ± 2.7	4.5 ± 0.04	76 ± 16	19 ± 3	459.6 ± 65	224 ± 89
Physiological saline	5W	(n=5)	—	—	314 ± 71	132 ± 16	1967 ± 236	740 ± 211
	6W	(n=1)	80<	2.6	162	49	1268	1011
H G F	50	(n=3)	32.0 ± 11.2	2.6 ± 0.8	165 ± 38	42 ± 14	1081 ± 523	789 ± 58
	200	(n=5)	21.4 ± 5.2	3.1 ± 0.7	164 ± 38	40 ± 12	1053 ± 448	666 ± 116

Example 4

Effect of HGF administration on improvement of liver fibrosis of rats induced with DMN

(1) Method

DMN was administered intraperitoneally to SD male rats (5 weeks-old) at a dose of 10μl/kg three consecutive days a week (Monday, Tuesday, Wednesday) for 4 weeks to make rats with liver fibrosis. To these rats was administered 1 mg/kg of HGF or 1 ml/kg of a solvent (phosphate buffered saline comprising 2.5 mg/ml of HSA and 0.01 % of Tween 80) five consecutive days a week (Monday, Tuesday, Wednesday, Thursday, Friday) from the initiation of DMN administration to the 4th day of the 4th week. On the 5th day of the 4th week, the rats were sacrificed, the livers were taken out and fixed in neutral formalin, and after preparation of sections, they were subjected to Masson's trichrome staining which stains fiber tissue and does not stain other tissue. Here, for comparison, the livers of normal rats were likewise stained. With respect to the liver tissue specimens of respective individuals, the area of fibrous tissue in the total area of the tissue section was calculated respectively by an image analysis apparatus (T. Watanabe et al. Analytical Cellular Pathology 4 (3), 248, 1992), and the degrees of fibrous tissue in normal tissue were compared. The samples used for the analysis includes 8 specimens of the normal rats, 8 specimens of the DMN + solvent administered group, and 6 examples of the DMN + HGF administered group (HGF + DMN) except for specimens of rats in which the neutralization activity for HGF was generated in their serum.

(2) Result

The results are shown in Fig. 8. Each point in the figure indicates the ratio (%) of fibrous tissue in an each individual liver of normal rats (n=8), DMN administered rats (n=8) or DMN + HGF administered rats (n=6). The horizontal bar indicates the average value.

As shown in Fig. 8, as compared with normal rats, the ratio of fibrous tissue in liver tissue of the DMN administered group increased to 7.3 % from 1.3 % (average, normal rats). On the other hand, in the case of the DMN + HGF administered group, the ratio decreased to 2.8 % compared to that in the DMN administration group. This phenomenon indicates the improvement of liver fibrosis induced with DMN by administration of HGF.

Claims

1. An agent accelerating collagen decomposition containing at least one of HGFs as an active ingredient.

2. The agent accelerating collagen decomposition according to claim 1, wherein the agent contains HGF as the HGFs.

3. A therapeutic agent for fibrosis disorder containing at least one of HGFs as an active ingredient.

4. The therapeutic agent for fibrosis disorder according to claim 3, wherein the fibrosis disorder is selected from the group consisting of arterial sclerosis, chronic glomerulonephritis, cutis keloid formation, progressive systemic sclerosis (PSS), liver fibrosis, pulmonary fibrosis, cystic fibrosis, chronic graft versus host disease, local and systemic scleroderma, Peyronie's disease, penis fibrosis, urethrostenosis after the test using a cystoscope, inner accretion

after surg ry, my lofibrosis and idiopathic retroperitoneal fibrosis.

5. The therapeutic agent for fibrosis disorder according to claim 3 or 4, wherein the agent contains HGF as the HGFs.

6. A method for accelerating collagen decomposition comprising administering at least one of HGFs in an effective amount.

7. A method for treating fibrosis disorder comprising administering at least one of HGFs in an effective amount.

8. Use of at least one of HGFs for producing an agent accelerating collagen decomposition.

9. Use of at least one of HGFs for producing a therapeutic agent for fibrosis disorder.

Fig. 1

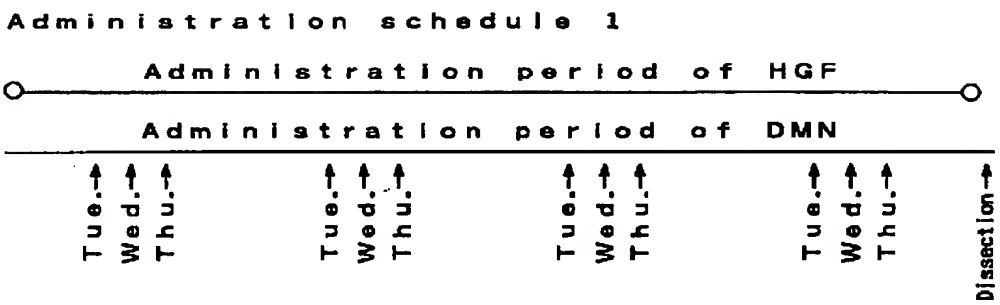


Fig. 2

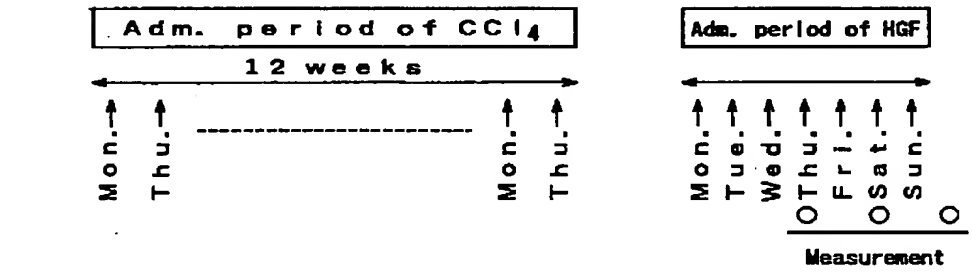


Fig. 3

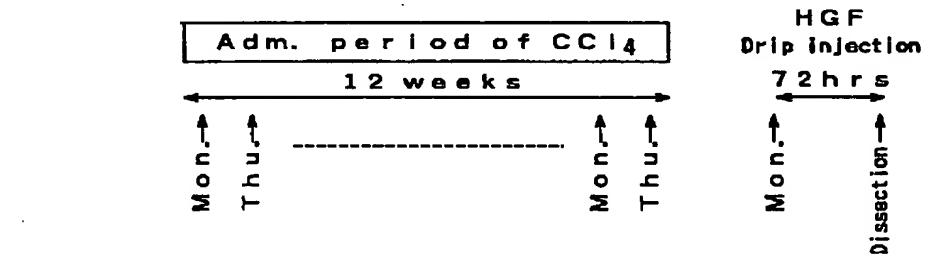


Fig. 4

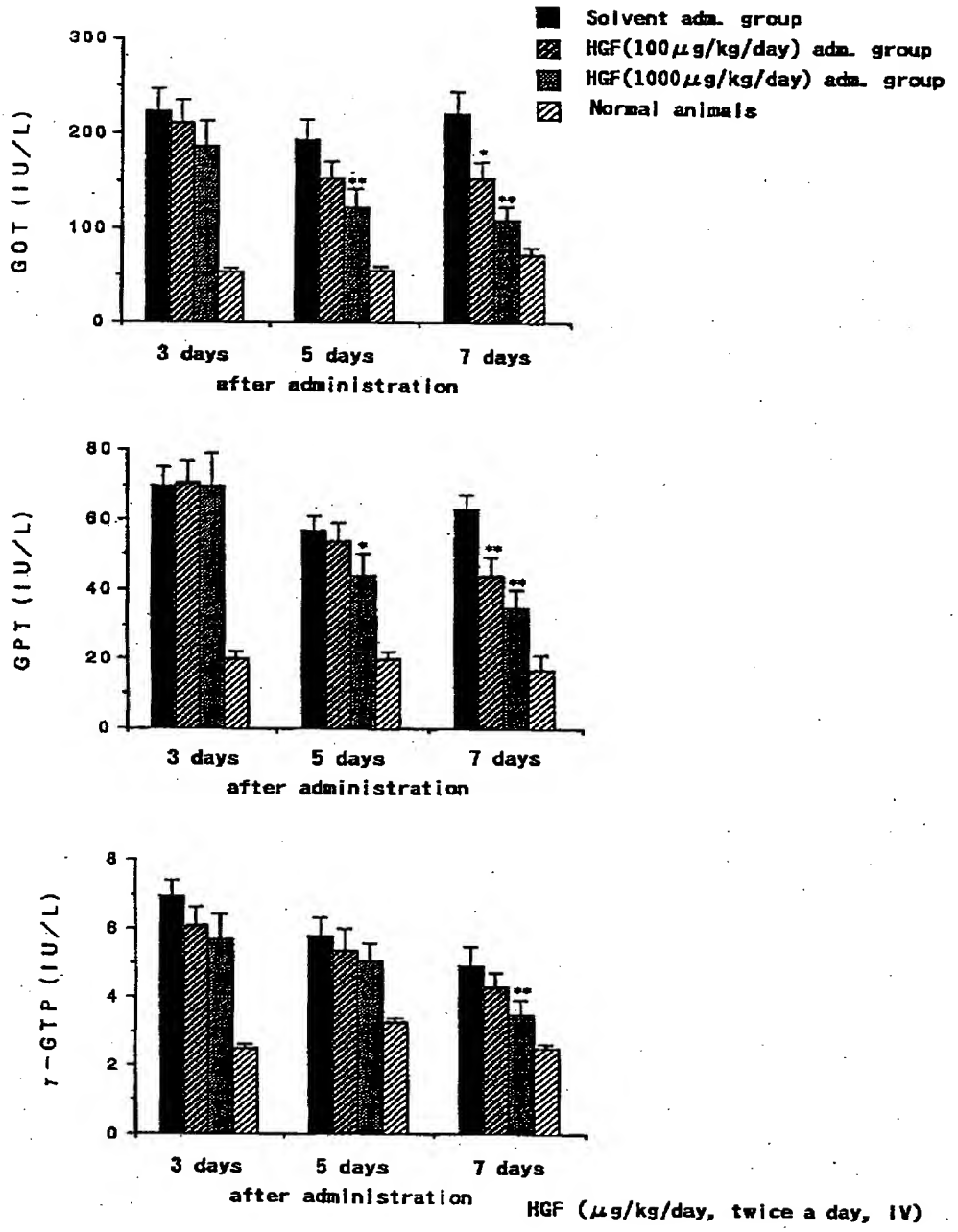

Solvent adm. group
HGF(100μg/kg/day) adm. group
HGF(1000μg/kg/day) adm. group
Normal animals
GOT (IU/L)
300
200
100
0
3 days
5 days
7 days
after administration
GPT (IU/L)
80
60
40
20
0
3 days
5 days
7 days
after administration
γ-GTP (IU/L)
8
6
4
2
0
3 days
5 days
7 days
after administration
HGF (μg/kg/day, twice a day, IV)

Fig. 5

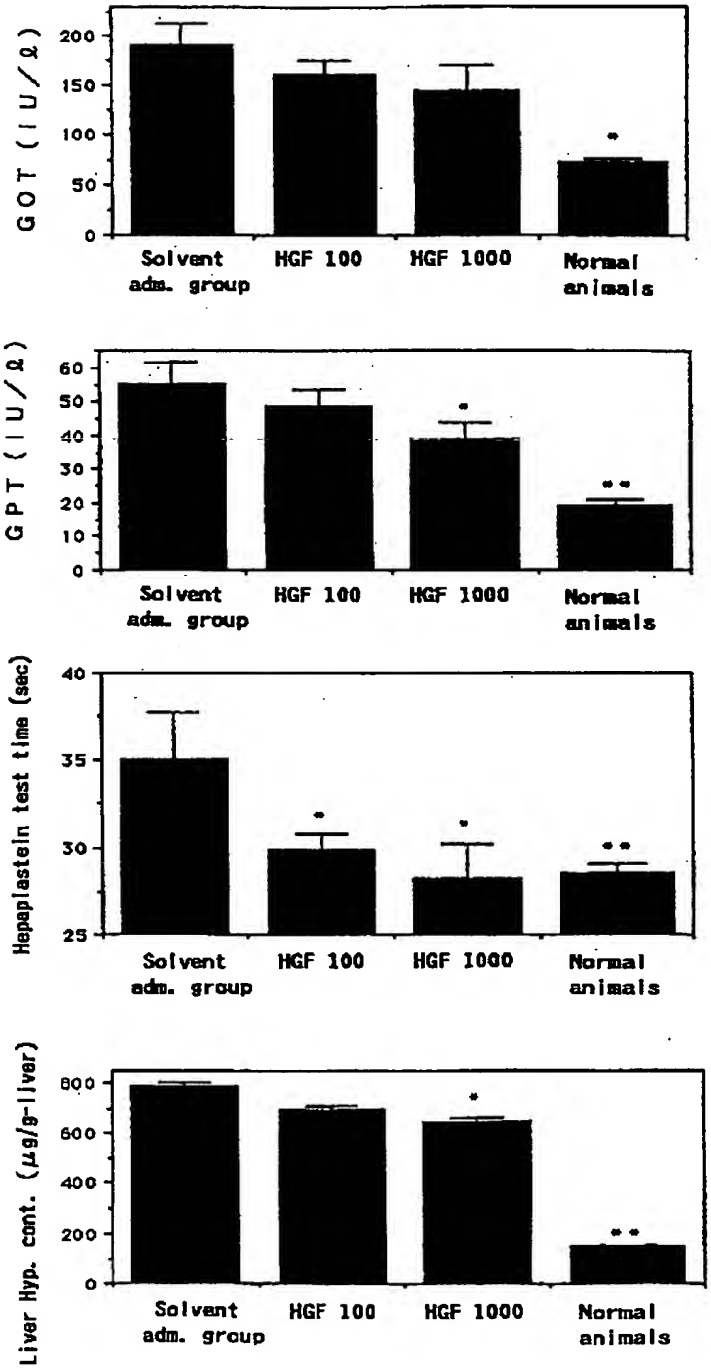

GOT (IU/ℓ)
200
150
100
50
0
*
Solvent adm. group
HGF 100
HGF 1000
Normal animals
GPT (IU/ℓ)
60
50
40
30
20
10
0
*
**
Solvent adm. group
HGF 100
HGF 1000
Normal animals
Hepaplastein test time (sec)
40
35
30
25
*
*
**
Solvent adm. group
HGF 100
HGF 1000
Normal animals
Liver Hyp. cont. (μg/g-liver)
800
600
400
200
0
*
**
Solvent adm. group
HGF 100
HGF 1000
Normal animals

Fig. 6

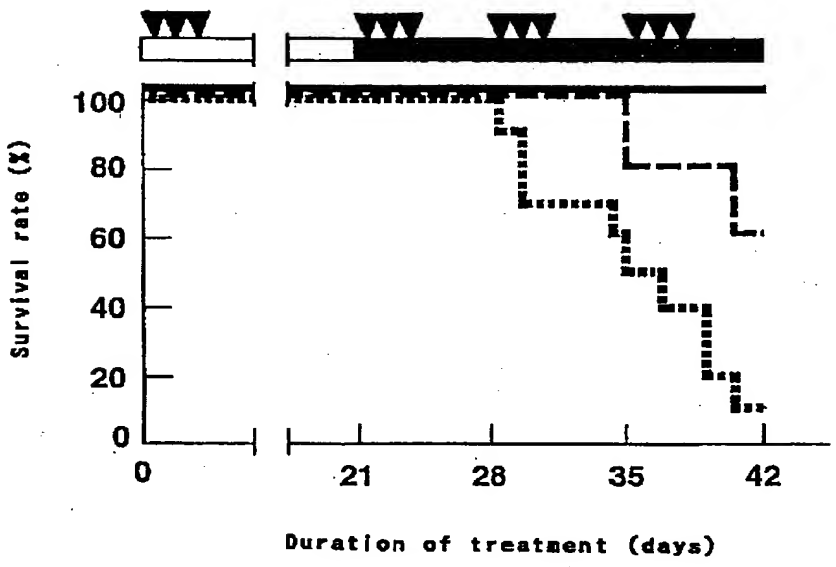

100
80
60
40
20
0
Survival rate (%)
0
21
28
35
42
Duration of treatment (days)

Fig. 7

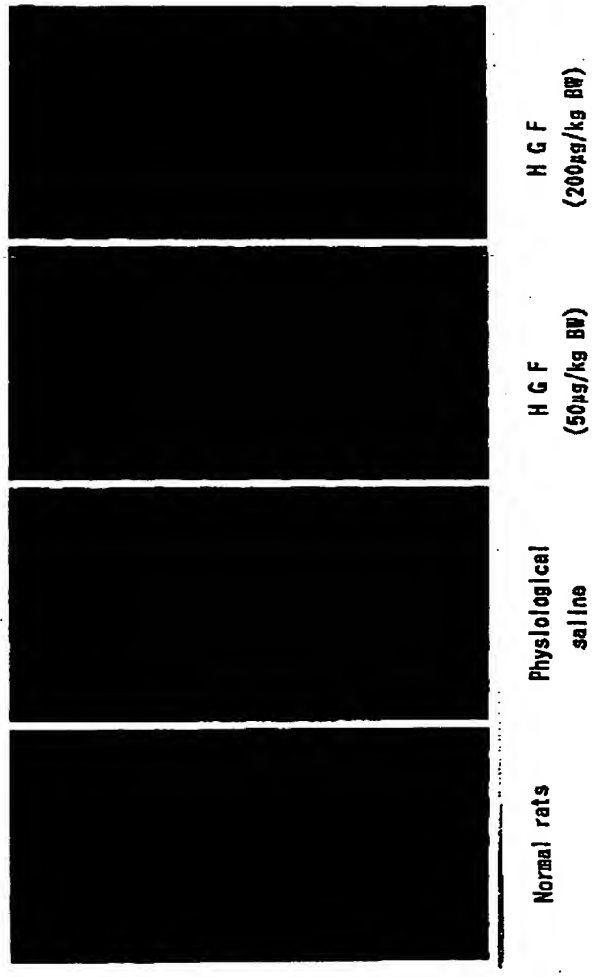
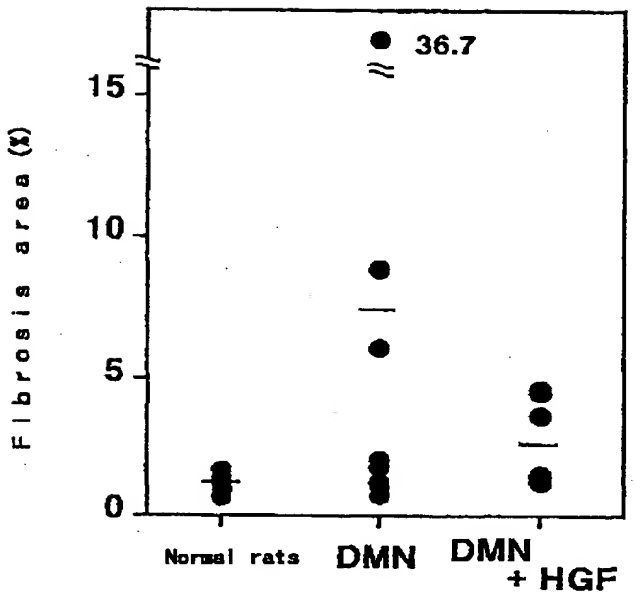

Normal rats
Physiological saline
HGF (50μg/kg BW)
HGF (200μg/kg BW)

Fig. 8



INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP95/00822

A. CLASSIFICATION OF SUBJECT MATTER
Int. Cl^{6} A61K38/18//C07K14/475

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
Int. Cl^{6} A61K38/18

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP, 492614, A (T. Nakamura et. al.), July 1, 1992 (01. 07. 92) &US, 5342831, A&JP, 7-179356, A	1-5, 8, 9
A	JP, 5-213733, A (Sansho Seiyaku K.K.), August 24, 1993 (24. 08. 93) (Family: none)	1-5, 8, 9
A	JP, 6-172207, A (T. Nakamura et. al.), June 21, 1994 (21. 06. 94) (Family: none)	1-5, 8, 9

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
July 18, 1995 (18. 07. 95)	August 8, 1995 (08. 08. 95)
Name and mailing address of the ISA/ Japanese Patent Office	Authorized officer
Facsimile No.	Telephone No.

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP95/00822

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 6, 7
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 6 and 7 pertain to methods for treatment of the human or animal body by therapy, and thus relate to a subject matter which this International Searching Authority is not required, under the provisions of Article 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under the PCT, to search.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1992)